**PATENT**
**ATTORNEY DOCKET NO.: DIVER1260-3**

Applicant: Short, et al.
Serial No.: 09/421,629
Filed: October 19, 1999
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D1 Delete

This application is a continuation application of U.S. Patent Application Serial No. 08/657,409, which was filed on June 3, 1996, now issued as U.S. Patent 5,958,672; which was a continuation-in-part of U.S. application Serial No. 08/568,994 which was filed on December 7, 1995, now abandoned; which is a continuation-in-part of U.S. application Serial No. 08/503,606 which was filed on July 18, 1995, now issued as U.S. Patent 6,004,788; and a continuation application of U.S. Patent Application Serial No. 09/089,789, filed June 3, 1998, pending; which is a continuation-in-part application of U.S. Patent Application Serial No.09/034,724, filed March 4, 1998, now issued as U.S. Patent No. 6,001,574; which is a continuation-in-part application of U.S. Patent Application Serial No. 08/665,565, filed June 18, 1996, now issued as U. S. Patent No. 5,763,239, each of which is incorporated herein by reference in its entirety.

The paragraph beginning on page 3, line 5 has been amended to read as follows:

Delete E2

The invention also provides a process of screening clones having DNA from an uncultivated microorganisms for a specified protein, e.g., enzyme, activity which comprises screening for a specified gene cluster protein product activity in the library of clones prepared by: (i) recovering DNA from a DNA population derived from at least one uncultivated microorganism; and (ii transforming a host with recovered DNA to produce a library of clones with the screens for the specified protein, e.g. enzyme, activity. The library is produced from gene cluster DNA which is recovered without culturing of an organism, particularly where the

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D2 Rule 83

DNA gene clusters are recovered from an environmental sample containing microorganisms which are not or cannot be cultured.

The paragraph beginning at line 25 of page 3 has been amended to read as follows:

D3 Rule 83

The microorganisms from which the libraries may be prepared include prokaryotic microorganisms, such as Eubacteria and Archeabacteria, and lower eukaryotic microorganisms such as fungi, some algae and protozoa. The microorganisms are uncultured microorganisms obtained from environmental samples and such microorganisms may be extremophiles, such as thermophiles, hypertherrnophiles, psychrophiles, psychrotrophs, etc.

The paragraph beginning at line 8 of page 5 has been amended to read as follows:

D4 Rule 84

Polyketides are molecules which are an extremely rich source of bioactivities, including antibiotics (such as tetracyclines and erythromycin), anti-cancer agents (daunomycin), immunosuppressants (FK506 and rapamycin), and veterinary products (monensin). Many polyketides (produced by polyketide syntheses) are valuable as therapeutic agents. Polyketide syntheses are multifunctional proteins, e.g. enzymes, that catalyze the biosynthesis of a hugh variety of carbon chains differing in length and patterns of functionality and cyclization. Polyketide synthase genes fall into gene clusters and at least one type (designated type I) of polyketide syntheses have large size genes and proteins, e.g. enzymes, complicating genetic manipulation and in vitro studies of these genes/proteins.

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The paragraph beginning at line 24 of page 5 has been amended to read as follows:

DS Drawings

Preferably, the gene cluster DNA is ligated into a vector, particularly wherein a vector further comprises expression regulatory sequences which can control and regulate the production of a detectable protein or protein-related array activity from the ligated gene clusters. Use of vectors which have an exceptionally large capacity for exogenous DNA introduction are particularly appropriate for use with such gene clusters and are described by way of example herein to include the f-factor (or fertility factor) of E. coli. This f-factor of E. coli is a plasmid which affect high frequency transfer of itself during conjugation and is ideal to achieve and stably propagate large DNA fragments, such as gene clusters from mixed microbial samples.

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The paragraph beginning at line 17 of page 6 has been amended to read as follows:

The following outlines a general procedure for producing libraries from nonculturable organisms, which libraries can be probed to select therefrom DNA sequences which hybridize to specified probe DNA:

Obtain Biomass
DNA Isolation
Shear DNA (25 gauge needle)
Blunt DNA (Mung Bean Nuclease)
Methylate (EcoR I Methylase)
Ligate to EcoR I linkers (GGAATTCC)
Cut back linkers (EcoR I Restriction Endonuclease)
Size Fractionate (Sucrose Gradient)
Ligate to lambda vector (Lambda ZAP7 (Stratagene) and gt11)
Package (in vitro lambda packaging extract)
Plate on E. [cold] coli host and amplify

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The paragraph beginning at line 1 of page 9 has been amended to read as follows:

D7 Amd E7

The screening for protein, e.g. enzyme, activity may be effected on individual expression clones or may be initially effected on a mixture of expression clones to ascertain whether or not the mixture has one or more specified protein, e.g. enzyme, activities. If the mixture has a specified protein, e.g. enzyme, activity, then the individual clones may be rescreened for such protein, e.g. enzyme, activity or for a more specific activity. Thus, for example, if a clone mixture has hydrolase activity, then the individual clones may be recovered and screened to determine which of such clones has hydrolase activity.

The paragraph beginning at line 1 of page 10 has been amended to read as follows:

D8 Amd E8

A particularly preferred type of vector for use in the present invention contains an f-factor origin of replication. The f-factor (or fertility factor) in E. coli is a plasmid which effects high frequency transfer of itself during conjugation and less frequent transfer of the bacterial chromosome itself. A particularly preferred embodiment is to use cloning vectors, referred to as "fosmids" or bacterial artificial chromosome (BAC) vectors. These are derived from the E. coli f-factor and are able to stably integrate large segments of genomic DNA. When integrated with DNA from a mixed uncultured environmental sample, this makes it possible to achieve large genomic fragments in the form of a stable "environmental DNA library."